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EVALUATION OF ANTIBACTERIAL ACTIVITY OF METAL NANOPARTICLES AGAINST DIFFERENT PATHOGENIC BACTERIA

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ABSTRACT

In recent years, infectious diseases are increasingly being encountered in clinical settings. Due to the development of antibiotic resistance and the outbreak of these diseases caused by resistant pathogenic bacteria, the pharmaceutical companies and the researchers are now searching for new unconventional antibacterial agents.

Recently, in this field, the application of nanoparticles is an emerging area of nanoscience and nanotechnology. For this reason, nanotechnology has a great deal of attention from the scientific community and may provide solutions to technological and environmental challenges. A common feature that these nanoparticles exhibit their antimicrobial behavior against pathogenic bacteria.

In this report, we evaluate the antibacterial activity of Ag, Fe and ZnO nanoparticles against both Gram-negative (E. coli and P. aeruginosa) and Gram-positive (Staph. aureus) bacteria, using agar well diffusion method, as well as determine of minimal bactericidal concentrations by the broth dilution method.

The results showed that antibacterial activities of these nanoparticles were found active against both Gram-positive and Gram-negative bacteria used in this study. Among the three nanoparticles, Ag nanoparticles have excellent bactericidal potential, while Fe nanoparticles exhibited the least bactericidal activity.

Keywords: Antibacterial activity, metal nanoparticles, Ag, ZnO, Fe.

I. INTRODUCTION

During the last decade, there has been an increasing interest in developing and understanding of matter at nanometric scale. The progress made in the area of nanotechnology in the last years has allowed the scientists to develop and characterize materials with outstanding properties and nanometric sizes. The field of nanomaterials has been the center of attention in the scientific community due to the unique and remarkable properties exhibited by materials when studied at nanometric scale [1].

A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm [2]. They possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications [3].

The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. Metal nanoparticles with bactericidal activity can be immobilized and coated on to surfaces, which may find application in various fields, i.e., medical instruments and devices, water treatment and food processing [4].

The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio i.e., large surface area of the nanoparticles enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities [5].

Among noble metal nanoparticles, silver nanoparticles have received considerable attention owing to their attractive physicochemical properties [6]. Ag-nanoparticles have already been tested in various field of biological science, drug delivery, water treatment and an antibacterial compound against both Gram (+) and Gram (-) bacteria by various researchers. Most of the bacteria have yet developed resistance to antibiotics and in this view in future it is need to develop a substitute for antibiotics [7]. Ag-nanoparticles are attractive as these are non-toxic to human body at low concentration and having broad-spectrum antibacterial nature [8]. According to the World Health Organization (WHO), silver (Ag) has little adverse effect on the health of human beings when the concentration is lower than the secondary minimum concentration level, which currently is 90 ppb. Ag-containing materials have been used for reducing infections in wound treatment, inhibiting bacteria colonization on many media, eliminating microorganisms on textile fabrics, and disinfecting potable water [9].

It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include 16 major species of bacteria. This aspect of silver makes it an excellent choice for multiple roles in the medical field [10].

The antibacterial effectiveness of the Ag-containing materials depends on many factors, such as the size and morphology of the Ag particles, the mechanical and chemical durability of the materials, and the release properties of the metallic Ag. Generally, the smaller the particle size is, the greater the antibacterial effectiveness is [9].

Nanoparticles were chosen because they can interact with microbial cells directly by disrupting/penetrating the cell envelope, produce secondary products, dissolved heavy metal ions, interrupting trans-membrane electron transfer, or indirectly being the carrier for other substances with antibacterial properties. Studies show that metals have proven to affect bacterial growth kinetics and have a high influence against bacterial strains growth [11].

Iron oxide (IO) has been widely used in biomedical research because of its biocompatibility and magnetic properties [12].

Spinel ferrite nanoparticles are of interest because of their well-known unique optical, electronic and magnetic properties. These nanoparticles have high permeability, good saturation magnetization, and no preferred direction of magnetization. They are magnetically “soft”, being easily magnetized and demagnetized, and electrically insulating. For these reasons, ferrites have been used as magnetic materials as well as refractory materials and catalysts [13].

Nano-sized particles of ZnO have more pronounced antimicrobial activities than large particles, since the small size (less than 100 nm) and high surface-to-volume ratio of nanoparticles allow for better interaction with bacteria. Recent studies have shown that these nanoparticles have selective toxicity to bacteria but exhibit minimal effects on human cells. ZnO nanoparticles have been shown to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria [14].

This work aimed at investigating the antibacterial activities of nanoparticles. For this purpose, spherical Ag, Fe, and ZnO NP were prepared using EEW technique. The structural properties of these nanoparticles are published elsewhere [15].

II. MATERIALS AND METHOD

Nanoparticles Preparations

Prior to the performing experiments, Ag, Fe, and Zn wires (dia: 0.25 mm; Purity: 99.998%; Alfa Aesar) and plates (dim: 20, 20, 1 mm; Purity: 99.998%; Alfa Aesar) were cleaned using emery paper and followed by Acetone. The wires each of 20 mm length were exploded on plates of the same material type in distilled water using the EEW set-up sketched in Fig. 1.

These wires have been exploded by bringing the metal wire into sudden contact with the metal plate. The above mentioned length of wire is standing for several sparks and is being replaced after several contacts.

Both the electrodes (wire and plate) are subjected to a potential difference of 36 V DC supplied from three 12 V batteries connected in series. The resulted NP remains suspended in water (colloidal form).

Independent experiments were performed to prepare each type of the nanoparticles. These nanoparticles were used to evaluate the antibacterial activity of Ag, Fe and ZnO nanoparticles against both Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*Staph. aureus*) bacteria

The EEW process is very energy intensive since only relatively low voltages are applied, and also results in large quantities of nanoparticles being produced from both the consumed electrodes.

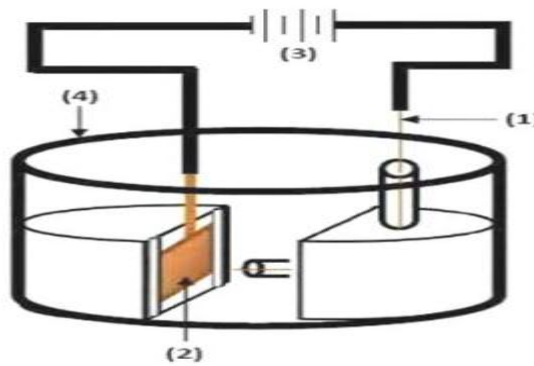

Figure-1 A schematic diagram of the electro-exploding wire (eew) set up; (1) Thin metal wire, (2) Metal plate, (3) 36 V batteries and (4) Glass vessel

Bactericidal Study

Clinical isolates of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus were obtained from laboratories of medical microbiology department, Ibb university, Yemen. All pathogenic bacteria were grown on nutrient agar plates and maintained in the nutrient agar slants at 4 °C for further use.

❖ **Determination of antibacterial activity by well-diffusion method**

The antibacterial activity of Ag, Fe and ZnO nanoparticles were tested against both Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*Staph. aureus*) bacteria, using the modified agar well diffusion method of [16]. In brief, the pure bacterial culture was sub-cultured in nutrient broth for 24 h at 37 °C. For bacterial growth, a lawn of culture was prepared by spreading the 100 µl fresh culture having approximately ($1.5x10^8$ CFU/mL) of each test organism on Mueller Hinton agar plates using sterile glass-rod spreader. Plates were left standing for 10 minutes to let the culture get absorbed. Then 6 mm wells were punched into the Mueller Hinton agar plates for testing nanoparticles antimicrobial activity with the help of a sterilized stainless steel cork borer. A 100 µL (50 µg) of nanoparticle suspension was poured onto each of three wells on all plates. The plates were incubated at 37 °C for 24 h. After incubation, the presence of bacterial growth inhibition zone around the sample loaded well was absorbed and their diameters (mm) were measured. Each nanoparticle was tested in triplicate with broad spectrum antibiotic gentamycin (10 µg/disc) as standard.

❖ **Determination of minimal bactericidal concentrations**

Minimum bactericidal concentration (MBC) for nanoparticles was determined by the broth dilution method, using the method of [17].

10 mL nutrient broth medium amended with 0 (control), 20, 40, 60, 80, 100, and 120 µg/mL nanoparticles was prepared separately. Each set was inoculated aseptically with 100 µL of respective bacterial strains suspension ($1.5x10^8$ CFU/mL), the inoculated sets were incubated at 37°C for 24 h. 100µl of the culture was plated on nutrient agar plates. The plates were incubated at 37°c for 24 hours. Viable bacterial colonies were counted and recorded by the naked eye determining, the lowest concentration that locked bacteria growth, defining this as the MBC. The experiments were carried out in triplicate, and the percentage of inhibition was calculated by the following formula:

$$\text{Inhibitory effect (\%)} = \frac{\text{No. of colonies in control - No. of colonies in test}}{\text{No. of colonies in control}} \times 100$$

III. RESULTS AND DISCUSSION

Antimicrobial properties

Antibacterial activity results revealed that Ag and ZnO nanoparticles acted as excellent antibacterial agents against both Gram-negative and Gram-positive bacteria when compared to Fe nanoparticles.

Ag nanoparticles exhibited maximum of inhibition zones (22 mm) bacterial growth inhibition against *Staph. aureus,* in the form of inhibition zones studies, where diffusion of nanoparticles on Mueller Hinton agar plates inhibits growth. In contrast, ZnO and Fe showed of inhibition zones 15 and 12 mm, respectively, against *Staph. aureus.*

In the case of *E. coli* maximum growth of inhibition zones were found to be the following; 17, 13, and 8 mm for Ag, ZnO and Fe respectively (Figure 2). Similar pattern was observed in the case of *P. aeruginosa* where the maximum of inhibition zones was exhibited by Ag followed by ZnO and Fe.

It appears that the antibacterial activity of the nanoparticles increased with increase in surface to volume ratio due to the decrease in size of nanoparticles.

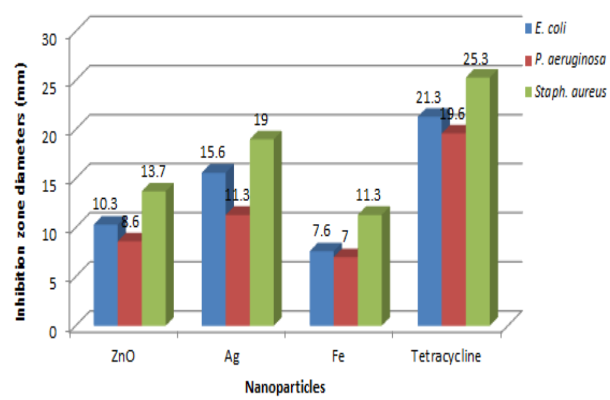

Figure-2 Bar graphs showing zone of inhibition introduced by different metal nanoparticles against various microorganisms

The minimum bactericidal concentration (MBC) representing the antimicrobial activity of Ag, ZnO and Fe nanoparticles dispersed in batch cultures is summarized in (fig 3, 4, 5). It was observed that the MBC for Ag nanoparticles are 50µg/ml for *Staph. aureus*, 60µg/ml for *E. coli* and *P. aeruginosa*. While the MBC for ZnO nanoparticles are 80µg/ml for

Staph. aureus, 100µg/ml for *E. coli* and *P. aeruginosa*. Whereas the MBC of Fe nanoparticles has been observed that the 120µg/ml for *Staph. aureus*, *E. coli* and *P. aeruginosa*.

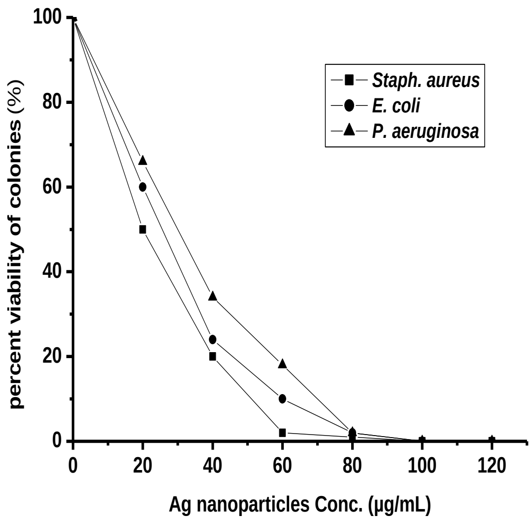

Figure-3 Minimum bactericidal concentration (MBC) of Ag nanoparticles against bacteria

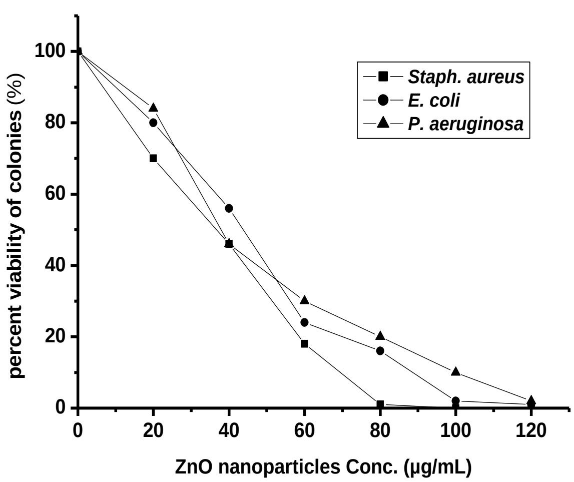

Figure-4 Minimum bactericidal concentration (MBC) of ZnO nanoparticles against bacteria

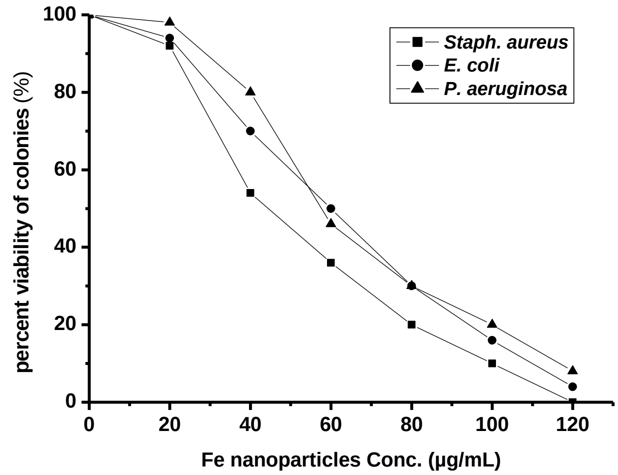

Figure-5 Minimum bactericidal concentration (MBC) of ZnO nanoparticles against bacteria

We demonstrated that the average of antibacterial activities of nanoparticles was Ag (15.30 nm) followed ZnO (10.87 nm) then Fe (8.63 nm), which indicates the size of the nanoparticles might also play a role in the antibacterial activity of each sample. Similar activity observations have been made for nanoparticles composed of a single metal oxide [18, 19, 20]. However, it should also be noticed that Gram-negative bacteria of *E. coli* and *P. aeruginosa* had inhibition-zone sizes that were lower than Gram-positive bacteria of *Staph. aureus* in the case of Ag nanoparticles. As well as in the case of ZnO and Fe nanoparticles, same Gram-negative bacteria of *E. coli* and *P. aeruginosa* had zone sizes that were lower than Gram-positive *Staph. aureus* bacteria. This observation could also be indicative of higher Gram-negative pathogenic bacteria resistance/tolerance against such nanoparticles over Gram positive pathogenic bacteria. Our finding is in agreement with [21, 22, 17] who reported that the Ag, ZnO, Fe nanoparticle effect is more pronounced against Gram-positive bacterial strains than Gram-negative bacterial strains.

Furthermore, previous studies have shown that the smaller the nanoparticle size, the greater the efficacy in inhibiting the growth of bacteria involving both the accumulation of nanoparticles and the production of reactive oxygen species. However, nanoparticles were previously reported to act both as bactericidal and bacteriostatic agents [18].

The minimum bactericidal concentration (MBC), defined as the lowest concentration of material that kills 99.9% of the bacteria was determined based on batch cultures containing varying concentration of Ag, ZnO and Fe nanoparticles in suspension [23].

The MBC values of Ag, ZnO and Fe against pathogenic bacteria were observed in the range of 10 - 120 mg/l, indicating very well bactericidal activity (represented by MBC) of the antibacterial agents (Fig. 2, 3, 4).

Since nanoparticles can be smaller in size than bacterial pores, they have a unique ability of crossing the cell membrane, disrupting its function or interfering with nucleic acid or protein synthesis [24]. The bactericidal pattern of our synthesized nanoparticles against both Gram-negative and Gram-positive pathogenic bacteria was again Ag, followed ZnO, and then Fe. Our results are supported by [21, 18] who reported that Ag and ZnO were the most toxic nanomaterial among ten other nanomaterials. Also our study is supported by [1], who reported that the Fe nanoparticles have been shown to exhibit the least bactericidal activity, when compared with other nanoparticles, like ZnO and CuO. The antibacterial activity of composite nanoparticles containing iron oxide, zinc oxide and zinc ferrite phases has been shown to be proportional with the weight ratio [Zn]/[Fe] of the composite nanoparticle, on *Staph. aureus* and *Escherichia coli*, so the lowest the iron concentration, the most intensive the antimicrobial activity [24]. Studies in the last decades on microbial adherence to different substrate led to the conclusion that the survival of microorganisms in the natural habitats, including medical ecosystems, is dependent on their capacity to adhere to different surfaces/substrata and form biofilms. A biofilm is a sessile microbial community composed of cells embedded in a matrix of extracellular polymeric substances attached to a substratum or interface. The matrix is primarily of microbial origin and the cell encased in this matrix present a modified phenotype, being metabolically more efficient and well protected, exhibiting resistance to different stress factors, including host defence mechanisms and antibiotics [25]. In this study, the concentration of nanoparticles was a major contribution to *Staph. aureus* activity inhibition. A similar concentration-dependent behavior was observed by [26] when they investigated the antimicrobial effects of Ag and ZnO nanoparticles on *Staph. aureus* and *E. coli*.

IV. CONCLUSION

Nanoparticles were show excellent bactericidal potential, therefore, we conclude that nan-biotechnology is an important area of research that deserves all our attention owing to its potential application to fight against multidrug-resistant microbes. Therefore, further studies must be conducted to assess the genotoxic and cytotoxic effects in human cells and environmental microorganisms in order to evaluate the applications of nanoparticles as a bactericidal agent.

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